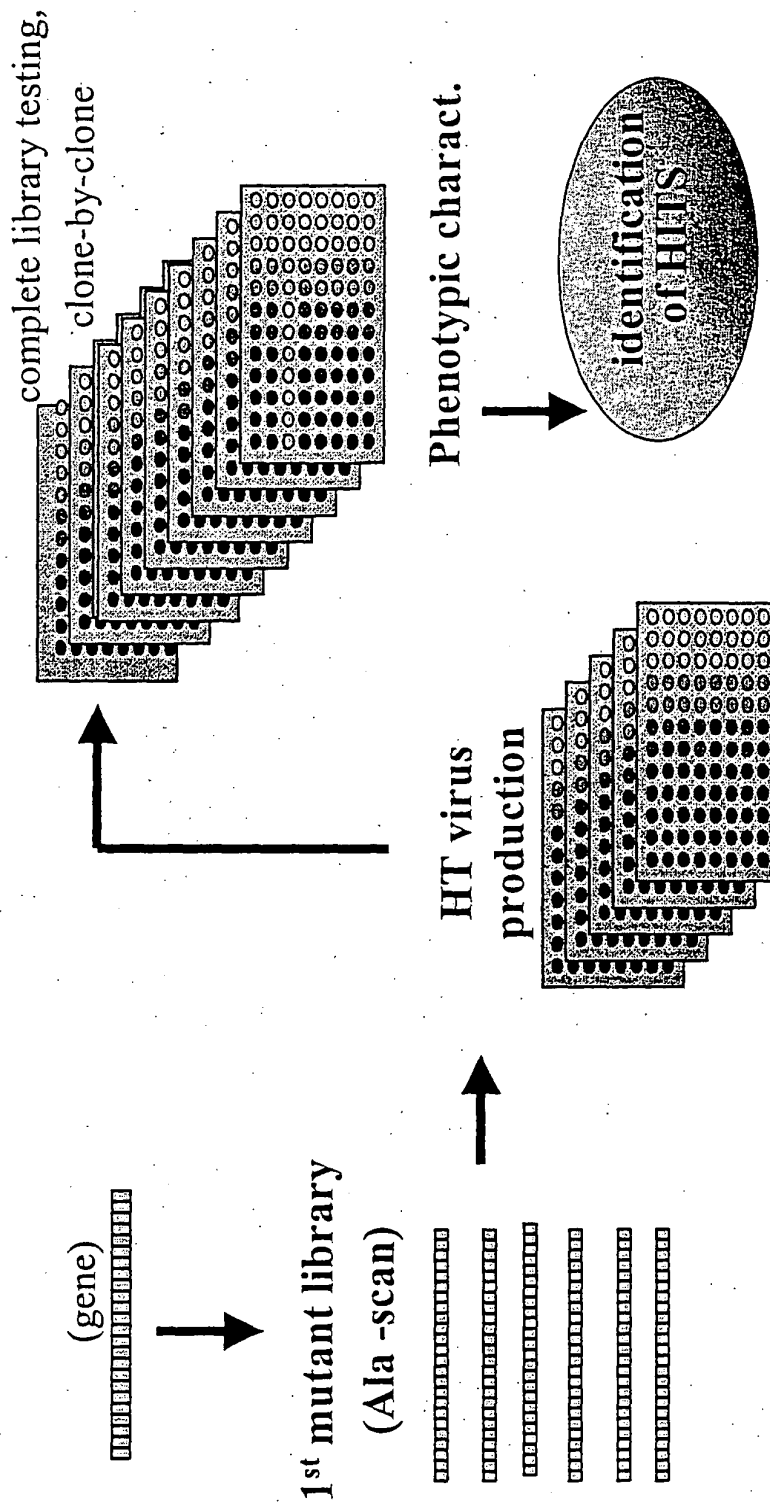


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#16. 1A

1st Round : screening of mutants (full length Ala-scan)



FOIA b 7 - D

FIG. 1B

2nd Round : screening of mutants at (surrounding) HIT positions

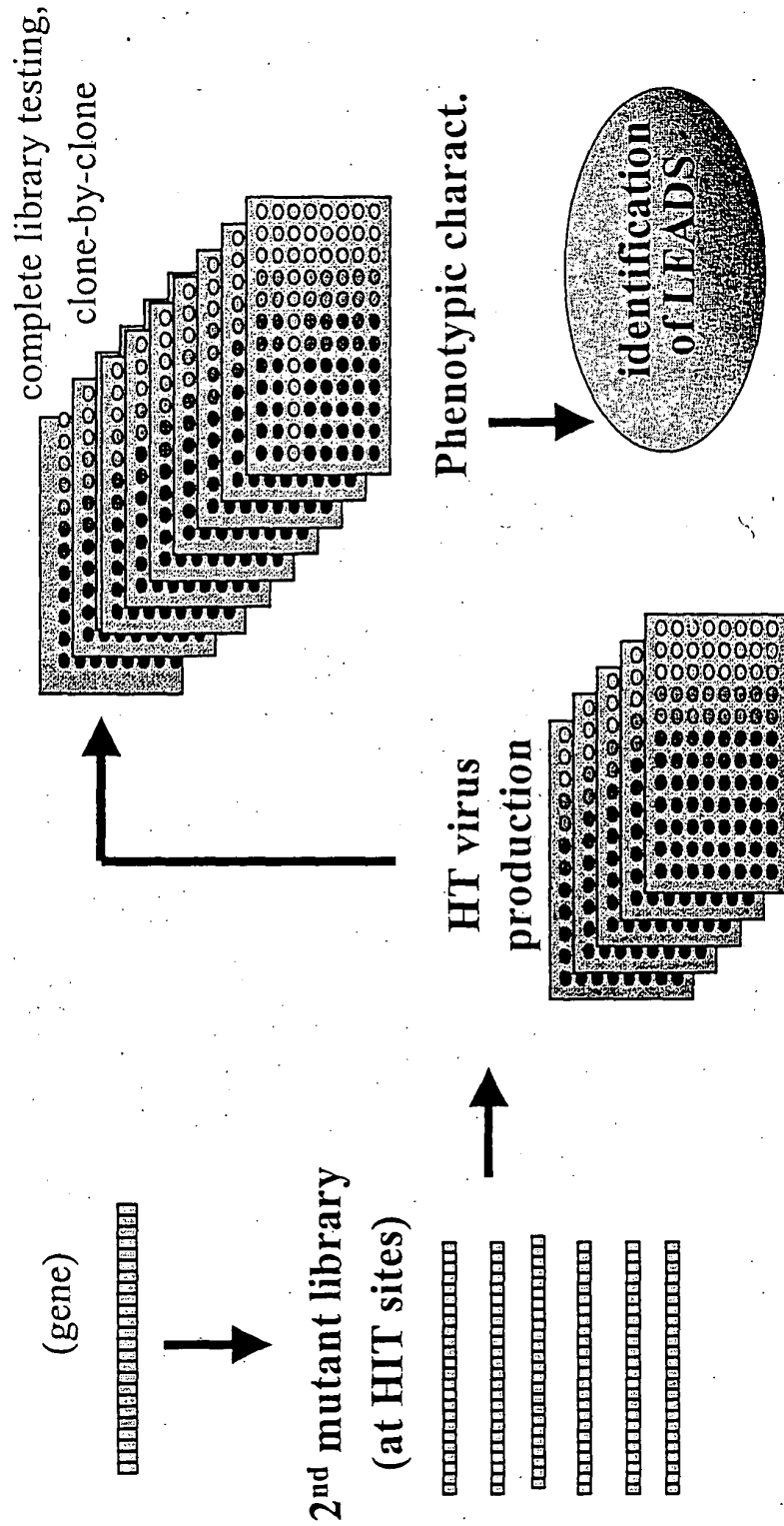


FIG. 1C

3rd Round : screening of recombinants between LEADS

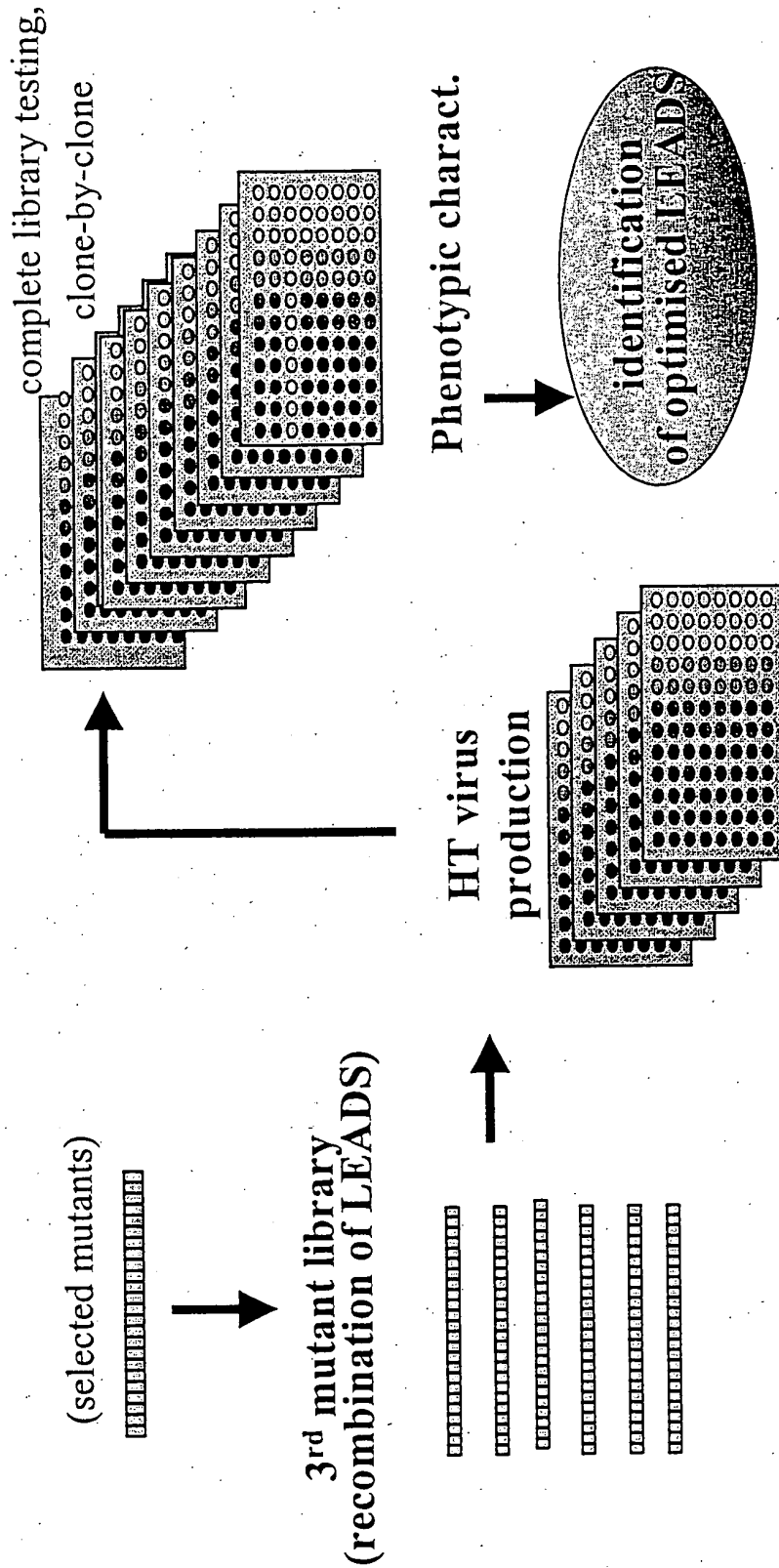
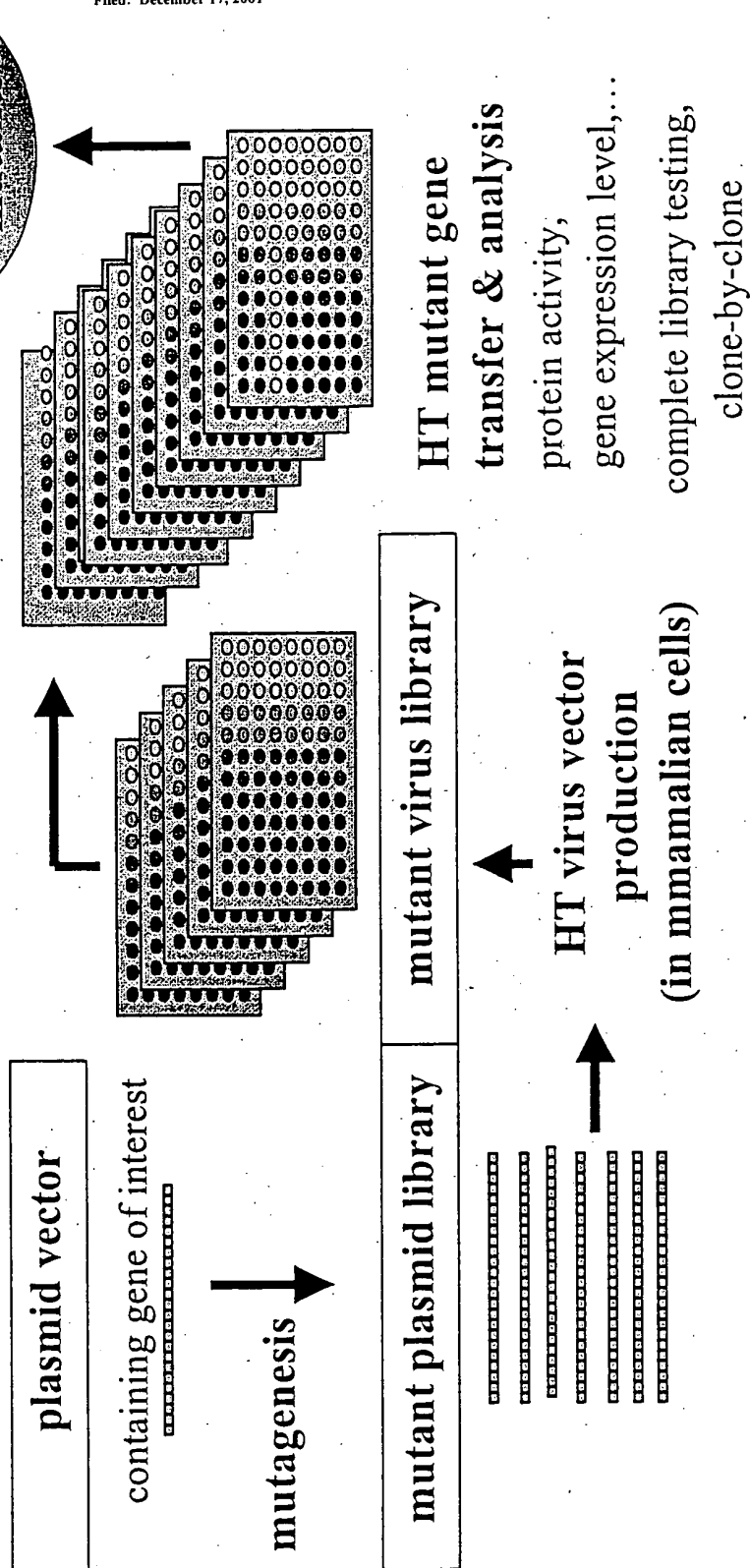


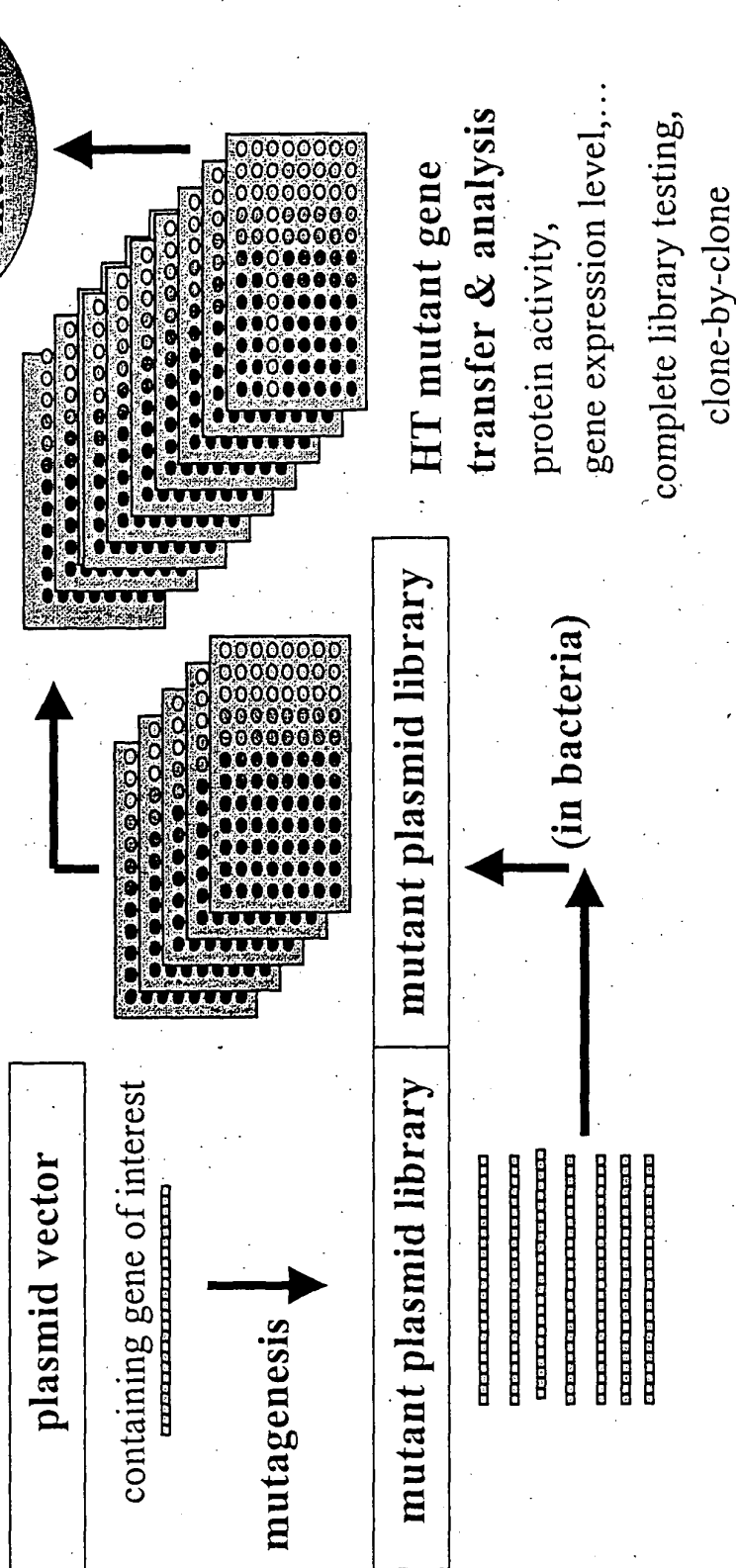
FIG. 1D

Gene optimisation (in mammalian cells)



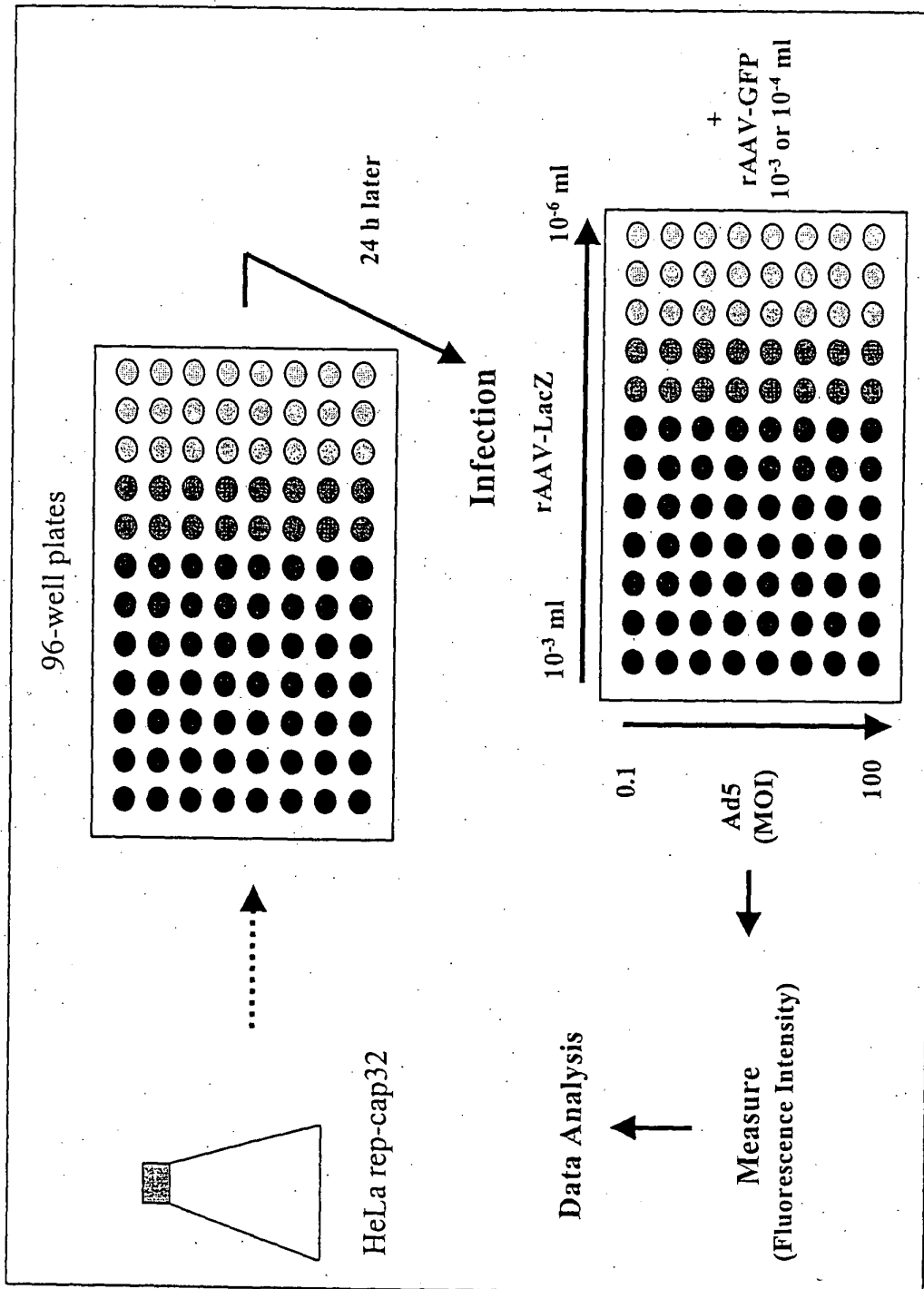
F16. 1E

Gene optimisation (in bacteria)



TOGETHER

FIG. 2A



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FIG 7B

TREE AAV lacZ 1 μ l+AAV GFP 1 μ l

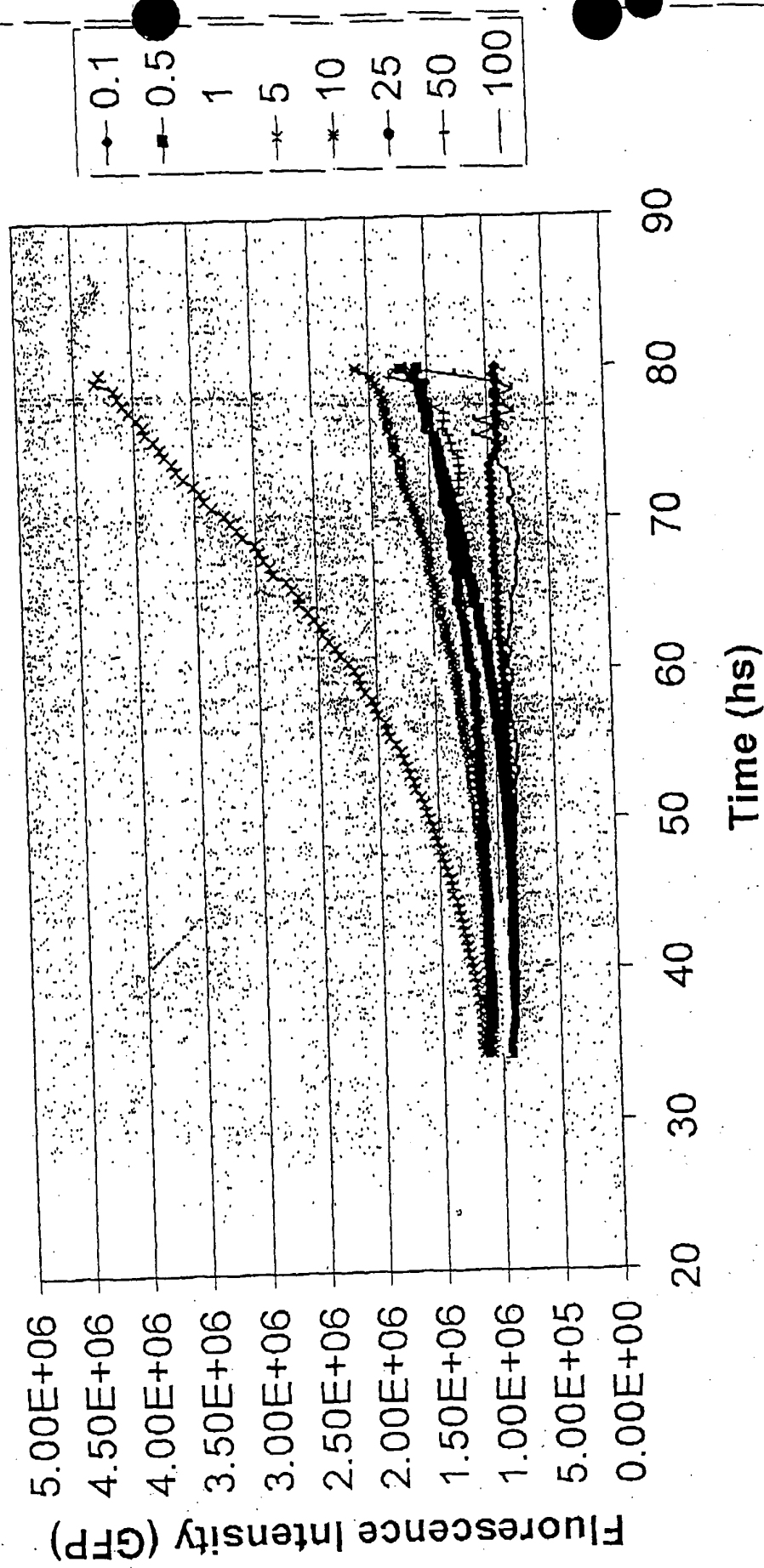


FIG. 2 C

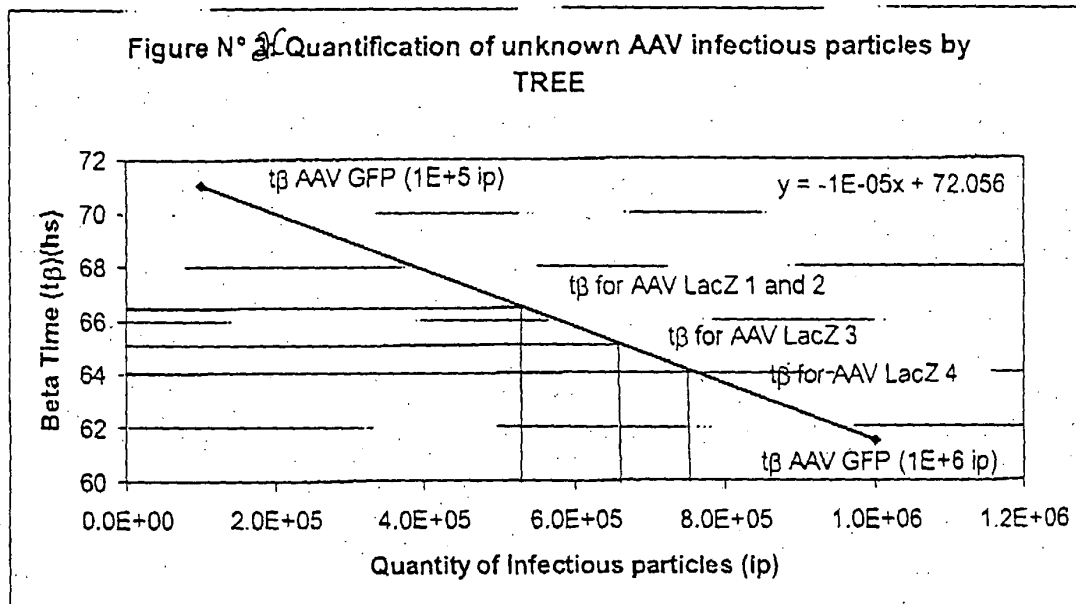


FIGURE 3A

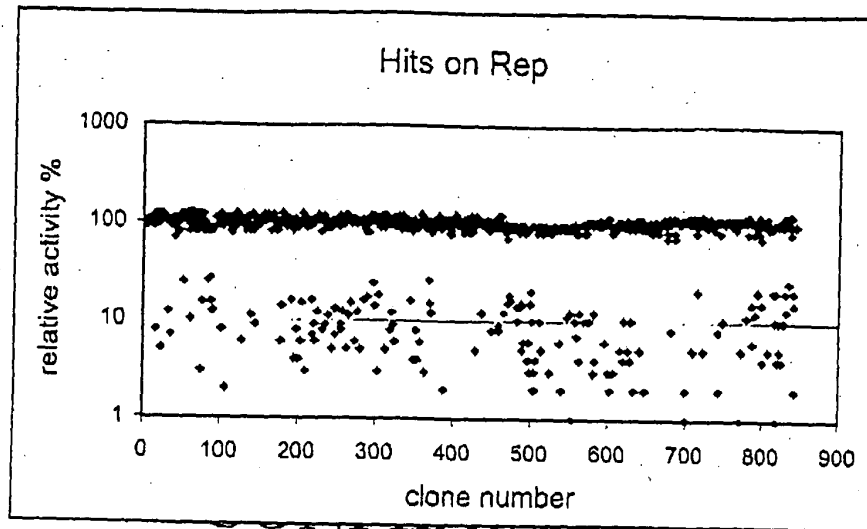


FIGURE 3B

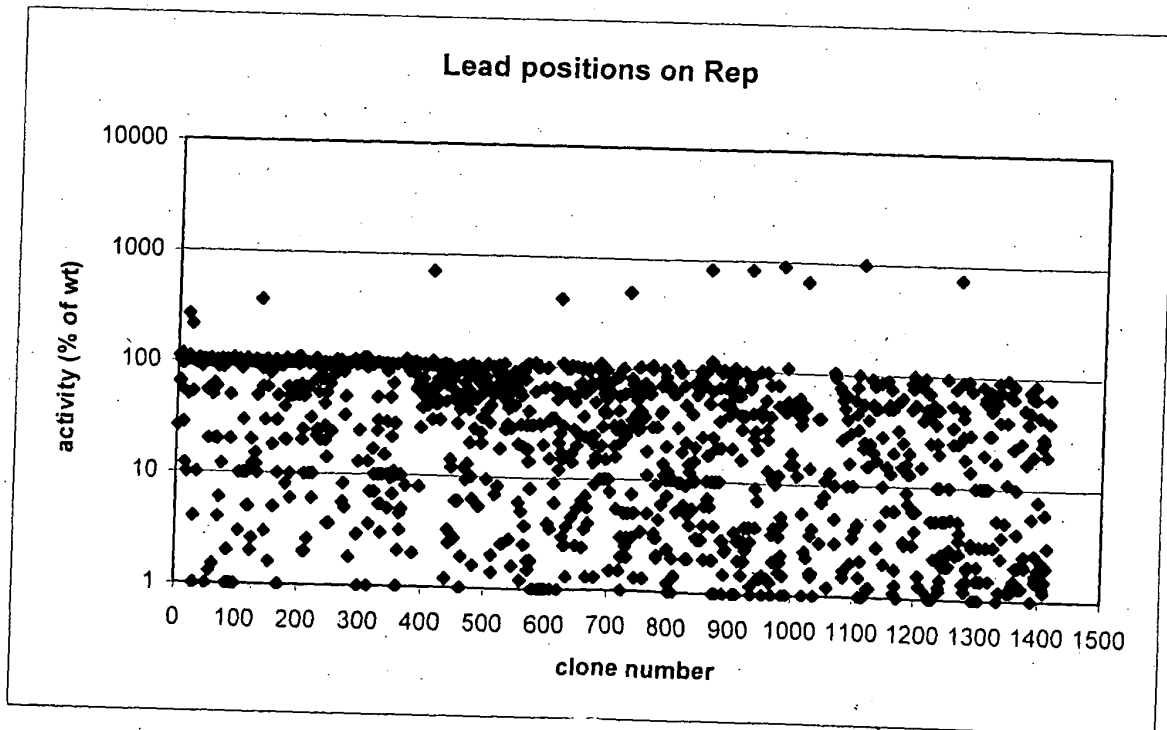
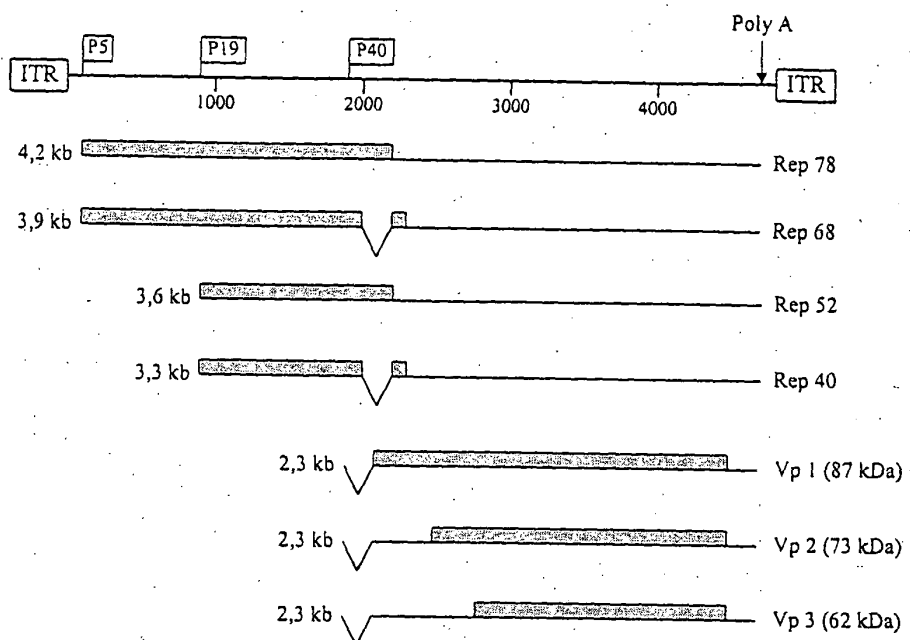


FIGURE 4



1002249-12101

FIGURE 5A

10 20 30 40 50 60
1 MPGFYEIVIKVPSDDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
2 MPGFYEIVIKVPSDDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
3 MPGFYEIVLKVPSDDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLIEQAPLTVAEKLQ 60
4 MPGFYEIVLKVPSDDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLIEQAPLTVAEKLQ 60
5 MPGFYEIVLKVPSDDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
6 MPGFYEIVIKVPSDDLGHLPGISDSFVNWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ 60
7 MATFYEIVIRVPFDVEEHLPGISDSFVDWVTGQIWELPPESDLNLTLVEQPQLTVADRIR 60
C M**FYE**:*VP*D**HLPGIS+SFV:WV****WELPP*SD**+*L*EQ**LTVA****

70 80 90 100 110 120
1 RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHILVETTGVKSMVLGRFLSQIRDKLVQTI 120
2 RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHILVETTGVKSMVLGRFLSQIRDKLVQTI 120
3 REFLVEWRRVSKAPEALFFVQFEKGETYFHLHVLTIETIGVKSMVVGRYVSIKEKLVTRI 120
4 REFLVEWRRVSKAPEALFFVQFEKGETYFHLHVLTIETIGVKSMVVGRYVSIKEKLVTRI 120
5 REFLVEWRRVSKAPEALFFVQFEKGDSYFHLHILVETVGKSMVVGRYVSIKEKLVTRI 120
6 RDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVKSMVLGRFLSQIREKLIQRI 120
7 RVFLYEWNKFSKQ-ESKFFVQFEKGEYFHLHTLVETSGISSMVLGRYVSIQIRALVKVV 119
C R:FL++W***SK**E**FFVQFEKG+:YFH*H:L+ET:G**SMV:GR::SQI::*L*::*

130 140 150 160 170 180
1 YRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECIIPNYLLPKTQPELQWAWTNMEEYISACL 180
2 YRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECIIPNYLLPKTQPELQWAWTNMEEYISACL 180
3 YRGVEPQLPNWFVAVTKTRNGAGGGNKVVDDCYIPNYLLPKTQPELQWAWTNMDQYLSACL 180
4 YRGVEPQLPNWFVAVTKTRNGAGGGNKVVDDCYIPNYLLPKTQPELQWAWTNMDQYLSACL 180
5 YRGVEPQLPNWFVAVTKTRNGAGGGNKVVDDCYIPNYLLPKTQPELQWAWTNMDQYISACL 180
6 YRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECIIPNYLLPKTQPELQWAWTNMEQYLSACL 180
7 FQGIPEQINDWVAITKVKK--GGANKVVDSGYIPAYLLPKVQPELQWAWTNLDEYKLAAL 177
C **G:EP:***W*A*TK*****GG*NKVV:D:*YIP*YLLPK*QPELQWAWTN*:Y:A*L

190 200 210 220 230 240
1 NLAERKRLVAQHLTHVSQTQEONKENLNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
2 NLAERKRLVAHDLTHVSQTQEONKENLNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
3 NLAERKRLVAQHLTHVSQTQEONKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
4 NLAERKRLVAQHLTHVSQTQEONKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
5 NLAERKRLVAQHLTHVSQTQEONKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK 240
6 NLTERKRLVAQHLTHVSQTQEONKENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK 240
7 NLEERKRLVAQFLAESSQRS-QEAASQREFSADPVIKSKTSQKYMALVNWLVEHGITSEK 236
C NL+ERKRLVA+L***SQ***Q****+***S**PVI*SKTS**YM*LV*WLV*+GITSEK

250 260 270 280 290 300
1 QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTSA PDYLVGPAPPADIKTNRIYR 300
2 QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTSA PDYLVGPAPPADIKTNRIYR 300
3 QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITKNRIYQ 300
4 QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITKNRIYQ 300
5 QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISSNRIYR 300
6 QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISSNRIYQ 300
7 QWIQENQESYLSFNSTGNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISKNRIWQ 296
C QWIQE*Q*SY*SFN***NSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI::NRI*:

310 320 330 340 350 360
1 ILELNGYEPAYAGSVFLGWAQKRFGRNTIWLFGPATTGKTNIAEIAHVPFYGCVNWT 360
2 ILELNGYDPAYAGSVFLGWAQKRFGRNTIWLFGPATTGKTNIAEIAHVPFYGCVNWT 360
3 ILELNGYDPQYASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEIAHVPFYGCVNWT 360
4 ILELNGYDPQYASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEIAHVPFYGCVNWT 360
5 ILEMNGYDPQYASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEIAHVPFYGCVNWT 360
6 ILELNGYDPQYASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWT 360
7 IFEMNGYDPAYAGSILYGWCQRSFNKRNTVWLYGPATTGKTNIAEIAHTVPFYGCVNWT 356
C I*E+NGY*P:YA:S***GW***:F*KRNT*WL*GPATTGKTNIAEIAH+VPFYGCVNWT

10022249-121701

	370	380	390	400	410	420
1	NENFPFNDCVDK	MVIWEEGKMTAKV	VESAKAILGGSKVR	VDQCKSSAQIDPT	TPVIVTS	420
2	NENFPFNDCVDK	MVIWEEGKMTAKV	VESAKAILGGSKVR	VDQCKSSAQIDPT	TPVIVTS	420
3	NENFPFNDCVDK	MVIWEEGKMTAKV	VESAKAILGGSKVR	VDQCKSSAQIEPT	TPVIVTS	420
4	NENFPFNDCVDK	MVIWEEGKMTAKV	VESAKAILGGSKVR	VDQCKSSAQIEPT	TPVIVTS	420
5	NENFPFNDCVDK	MVIWEEGKMTAKV	VESAKAILGGSKVR	VDQCKSSAQIDPT	TPVIVTS	420
6	NENFPFNDCVDK	MVIWEEGKMTAKV	VESAKAILGGSKVR	VDQCKSSAQIDPT	TPVIVTS	420
7	NENFPFNDCVDK	MLIWEEGKMTNKN	VESAKAILGGSKVR	VDQCKSSVQIDST	TPVIVTS	416
C	NENFPFNDCVDK	M*IWEEGKMT*KV	VESAKAILGGSKVR	VDQCKSS*QI+*T	TPVIVTS	

	430	440	450	460	470	480
1	NTNMCAVIDGNSTTFEHQOPLQDRMFKFELTRRLEHDFGKVTQKEVKEFFRWAQDHSVTEV					480
2	NTNMCAVIDGNSTTFEHQOPLQDRMFKFELTRRLEHDFGKVTQKEVKEFFRWAQDHSVTEV					480
3	NTNMCAVIDGNSTTFEHQOPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWASDHVTDV					480
4	NTNMCAVIDGNSTTFEHQOPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWASDHVTDV					480
5	NTNMCAVIDGNSTTFEHQOPLQDRMFKFELTRRLEHDFGKVTQKEVKDFFRWASDHVTEV					480
6	NTNMCAVIDGNSTTFEHQOPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWAKDHVVEV					480
7	NTNMCVVVDGNSTTFEHQOPLQDRMFKFELTRRLPDPFGKLTQKEVKDFFAWAKVNPVPV					476
C	NTNMC*V* DGNSTTFEHQOPL *DRMFKFELT+RL: *DFGK*TKQEVK+FF*WA: ***+: V					

	490	500		510	520
1	AHEFYVRKGGANKRPAPDDADKSEPKRA-----			CPSVADPSTSDAEG	522
2	AHEFYVRKGGANKRPAPDDADKSEPKRA-----			CPSVADPSTSDAEG	522
3	AHEFYVRKGGAKKRPA NS DADVSEPKRQ-----			CTSLAQPTTSDAEA	522
4	AHEFYVRKGGAKKRPA NS DADVSEPKRQ-----			CTSLAQPTTSDAEA	522
5	THEFYVRKGGARKRPAPNDADISEPKRA-----			CPSVAQPSTSDAEA	522
6	EHEFYVKGGAKKRPA PS DADISEPKRV-----			RESVAQPSTSDAEA	522
7	THEFKVPRELAGTKGA E SKSLRPLGDVTNTSYKSLEKRLR L SVFPETPRSSDVTVDPA L				536
C	:HEF*V+***A:***A:****:*****:			+:*::*:***A*:	

	530	540	550	560	570	580
1	APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFP--GVSESQ	580				
2	APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFP--GVSESQ	580				
3	P-ADYADRYQNKCSRHVGMNLMFLPCKTCERMNQISNVCFTHGQDCGECFPGMSQSPV	581				
4	P-ADYADRYQNKCSRHVGMNLMFLPCKTCERMNQISNVCFTHGQDCGECFPGMSQSPV	581				
5	P-VDYADRYQNKCSRHVGMNLMFLPCKQTCERMNQNVDFCFTHGVMDCAEFCF--VSESQPV	580				
6	S-INYADRYQNKCSRHVGMNLMFLPCKQTCERMNQNSNICFTHGQKDCLECFP--VSESQF	579				
7	RPLNWNSTRYDCKCDYHAQFDNISNKKDCEYLNRRGKNGCICHNVTHCQICHG-----	588				
C	:::+:**RY**KC**H:::****C:::CE*N*:::*.C**H*::*.C.*C**..:::+::::					

	590	600	610	620	
1	PVVRKRTYRKLCAIHLL GRAPE IIAC SAC DLVNVDLDDCVSEQ				623
2	PVVRKRTYRKLCAIHLL GRAPE IIAC SAC DLVNVDLDDCVSEQ				623
3	SVVKKKTYQKLCPIHHIL GRAPE IIAC SAC DLVNVDLDDCVSEQ				624
4	SVVKKKTYQKLCPIHHIL GRAPE IIAC SAC DLANVDLDDCVSEQ				624
5	SVVRKRTYQKLCPIHHIM GRAPE VAC SAC ELANVDLDDC DME Q				623
6	VSVVKKAYQKLCYIH HIMG -KVPD ACTAC DLVNVDLDDC IF EQ				621
7	----- IP W EKEN LS DFG DFD DAN KEQ				610
C	:+:+:+:*:*:*:+:++++*:++*:*:D*DD*:EQ				